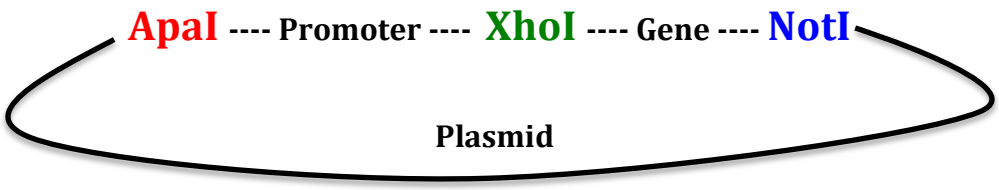
Restriction Enzyme Digestion

Our shuttle vector design is such that there are different enzyme cut sites between each of our fragments:



This means we will set up a different digestion reaction for each DNA "part". To find more information about the enzymes and what temperatures they require, visit the New England Biolabs website (www.neb.com).

Promoter digest:

1. Add 5µl CutSmart Buffer to your PCR tube.
2. Add 0.5 µl **ApaI** restriction enzyme to the tube. Vortex to mix well.
3. Incubate at room temperature for at least 1 hour.

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4. Add 0.5 µl **XhoI** restriction enzyme to the tube. Vortex to mix well.
5. Incubate at 37° for at least 1 hour.

Gene digest:

1. Add 5µl CutSmart Buffer to your PCR tube.
2. Add 0.5 µl **XhoI** and 0.5 µl **NotI** restriction enzyme to the tube. Vortex to mix well.
3. Incubate at 37° for at least 1 hour.

Plasmid digest:

1. Take 10 µl of plasmid from the plasmid stock (pJW608) into a new tube.
2. Add 3µl CutSmart Buffer and 16 µl of water to your tube.
3. Add 0.5 µl **ApaI** restriction enzyme to the tube. Vortex to mix well.
4. Incubate at room temperature for at least 1 hour.

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5. Add 0.5 µl **NotI** restriction enzyme to the tube. Vortex to mix well.
6. Incubate at 37° for at least 1 hour.